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THE SEROLOGIC DIAGNOSIS OF LEPROSY *

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It is our intention to answer, if possible, the following questions bearing on the serologic diagnosis of leprosy.

1. To what extent does the serum of a leper react in complement fixation with tuberculin, about which much has been written?
2. What relation does this phenomenon bear to the Wassermann reaction?
3. What attitude does tuberculin show toward non-leprous serum in regard to complement fixation?
4. Does or does not the leprous blood contain some antigens produced by the bacilli in the body? That is, may the blood contain such properties as have arisen from the bacilli themselves, or the products of the organic cells produced by the irritant effect of the bacilli?
5. Might these properties remain inactive in complement-fixation tests because of the immune properties produced by such antigens?
6. Will such properties be shown, as a result of comparative study, to be present in the blood of a patient suffering from diseases other than leprosy?

EXPERIMENTS

The following materials were employed in our experiments:

Antigen.—1. Koch's old tuberculin (prepared by the Imperial Institute for the Study of Infectious Diseases, Tokio). Each examination was made with 0.2 c.c. of the dilution in the proportions of 1 : 20, 1 : 40, and sometimes 1 : 10.

2. Alcoholic extract of the heart of a guinea-pig, diluted with physiologic salt solution. The heart was washed in physiologic salt solution and cut up until it presented a pasty appearance, to which twenty volumes of 90 percent alcohol were added. This was allowed to stand for two or more days at room temperature, being shaken at intervals only. Before use it was evaporated at 37 C. The remaining sticky mass was made into a suspension with six volumes of 0.85 percent sterile salt solution, to which carbolic acid in the proportion of 0.5 percent was added. It was then thoroughly shaken and a slightly whitish solution obtained. Of this original solution, 0.4 c.c. was used as the standard quantity, which was diluted sufficiently to avoid auto-inhibition. For the sake of convenience, this may be called Wassermann's syphilis antigen.

3. Leprous nodule. Leprous cutaneous nodules were taken out, the adherent connective and fatty tissue removed, and the remainder washed in physiologic salt solution and cut up until reduced to a pasty mass. It was then treated in the same manner as the guinea-pig heart.

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4. Clot of blood. (*a*) A clot of leprous blood, which was separated from a serum that had given a positive reaction with leprous antigen, and which remained quite free from the wall of the sterile tube containing the blood while the separation of the serum and clot was taking place, was removed after complete separation of the serum had been effected by standing it for twenty-four to forty-eight hours at room temperature or for two hours in an incubator. The clot was then washed in 0.85 percent salt solution, and cut up into a pasty mass. It was then treated exactly like the heart extract. For the sake of convenience, this may be termed the "leprous positive clot antigen."

(*b*) A clot of blood, the serum of which was negative in the complement-fixation test with leprous antigen, was treated in the same manner as the clot in (*a*) and the product called the "leprous negative clot antigen."

(*c*) A clot of blood from a non-leprous patient (i. e., syphilitic) which had given negative results in the complement-fixation test with the leprous nodule antigen, was treated in the manner described in (*a*) and the product called the "non-leprous clot antigen."

(*d*) A clot of blood from a guinea-pig was treated in the same manner and the product called the "guinea-pig clot antigen."

Salt Solution.—A normal solution in the proportion of 0.85 percent was employed.

Complement.—Fresh serum from a normal guinea-pig.

Hemolytic Amboceptor.—Serum of a rabbit that had been immunized with goat corpuscles, and which was proved to have a hemolytic efficiency as high as 2,000 to 4,000, was made into a dilution having three times the strength of the minimal efficiency.

Corpuscles.—Goat corpuscles were centrifugated three times and washed with salt solution and then a suspension of twenty times the volume of the original blood was made with normal salt solution. These were treated according to the following method: The dilute solution of hemolytic amboceptor and the corpuscle suspension were mixed in equal parts, and after thorough stirring the mixture was kept for thirty minutes at 37 C., when it was ready for use as a hemolytic system. One cubic centimeter was used for each test.

The test may be tabulated as follows:

TABLE 1

COMPLEMENT-FIXATION TEST

Test Tubes	Diluted Serum of Patient	Antigen	Ten Parts Dilution of Complement	Additional Salt Solution	Hemolytic System
Control 1	0.5 (0.2)	...	0.5	0.5	1.0
Experiment 1 ...	0.5 (0.1)	0.2	0.5	0.3	1.0
Experiment 2 ...	0.5 (0.05)	0.2	0.5	0.3	1.0
Control 2	....	0.4	0.5	0.6	1.0
Control 3	....	...	0.5	1.0	0.5
Control 4	....	...	...	1.5	1.0

The figures in parentheses in the column of patient's blood serum show the total amounts of the serum.

All the preparations were mixed and incubated together for one hour at 37 C. before adding the hemolytic system. After the addition of the hemolytic system, they were kept at 37 C. for one hour, after which they were shaken thoroughly and then again left for one hour and the results recorded.

TABLE 2—EXPERIMENTS

Patients			Antigens and Degrees of Reactions													
			Old Tuberculin		Wassermann's Syphilis Antigen		Leprous Nodule Antigen		Leprous Positive Clot Antigen		Leprous Negative Clot Antigen		Non-leprous Clot Antigen		Guinea-pig Clot Antigen	
			1	2	1	2	1	2	1	2	1	2	1	2	1	2
1.	M.	Severe tubercular leprosy	+++	++	......	......	++	++	++++	++++	....	....	—	—	++++	++++
2.	F.	Severe tubercular leprosy	++	++	+++++	+++++	+++	+++	+++++	+++++	—	—	—	—	......	......
3.	M.	Severe anesthesic leprosy	++++	++++	+++++	+++++	+++	+++	+++++	+++++	+	+	—	—	......	......
4.	M.	Light anesthesic leprosy	—	—	......	......	—	—	—	—	—	—	—	—	......	......
5.	F.	Severe anesthesic leprosy	++	++	......	......	—	—	+	+	—	—	—	—	......	......
6.	M.	Light tubercular leprosy	—	—	......	......	—	—	+	+	....	....	—	—	......	......
7.	M.	Mild anesthesic leprosy..	—	—	......	......	—	—	—	—	....	....	...	....	—	—
8.	M.	Mild tubercular leprosy..	++	+	......	......	+++	+++	+++++	+++++	....	....	...	....	+++++	+++++
9.	F.	Light anesthesic leprosy	—	—	......	......	—	—	—	—	....	....	...	....	—	—
10.	M.	Severe anesthesic leprosy	—	—	......	......	—	—	—	—	....	....	...	....	—	—
11.	M.	Mild anesthesic leprosy..	—	—	......	......	—	—	—	—	....	....	...	....	......	......
12.	M.	Severe tubercular leprosy	+++++	+++++	......	......	+++	+++	+++++	+++++	....	....	—	—	......	......
13.	M.	Light anesthesic leprosy	+	+	......	......	++++	++++	......	......	—	—	...	....	......	......
14.		Mild tubercular leprosy..	—	—	......	......	++++	++++	......	......	—	—	...	....	......	......
15.		Light anesthesic leprosy	+	+	......	......	—	—	+	+	....	....	...	....	......	......
16.	M.	Light tubercular leprosy	+++	+++	......	......	......	......	......	......	—	—	—	—	......	......
17.	M.	Mild anesthesic leprosy..	—	—	......	......	......	......	......	......	—	—	—	—	......	......
18.	M.	Light tubercular leprosy	—	—	......	......	......	......	......	......	—	—	—	—	......	......
19.	M.	Severe tubercular leprosy	+++++	+++++	+++++	+++++	......	......	......	......	....	....	...	....	......	......
20.	M.	Severe tubercular leprosy	++++	++++	—	—	......	......	......	......	....	....	...	....	......	......
21.	M.	Mild tubercular leprosy..	+++	+++	—	—	......	......	......	......	....	....	...	....	......	......
22.	M.	Mild tubercular leprosy..	+++	+++	—	—	......	......	......	......	....	....	...	....	......	......
23.	M.	Severe tubercular leprosy	+++++	+++++	+++++	+++++	......	......	......	......	....	....	...	....	......	......
24.	M.	Severe anesthesic leprosy	+	+	++++	++++	......	......	......	......	....	....	...	....	......	......
25.	M.	Light tubercular leprosy	+++	+++	—	—	......	......	......	......	....	....	...	....	......	......
26.	M.	Severe tubercular leprosy	++++	++++	++++	++++	......	......	......	......	....	....	...	....	......	......
27.	F.	Mild tubercular leprosy..	++	+	—	—	......	......	......	......	....	....	...	....	......	......
28.	M.	Severe tubercular leprosy	+	+	++++	++++	......	......	......	......	....	....	...	....	......	......
29.	F.	Severe tubercular leprosy	+++	++	++++	++++	......	......	......	......	....	....	...	....	......	......
30.	F.	Severe tubercular leprosy	+++	++	++++	++++	......	......	......	......	....	....	...	....	......	......
31.	M.	Light anesthesic leprosy	+	+	++++	++++	......	......	......	......	....	....	...	....	......	......
32.*	M.	Severe tubercular leprosy	++++	++++	+++	+++	......	......	......	......	....	....	...	....	......	......
33.	F.	Severe tubercular leprosy	+++	++	+++	+++	......	......	......	......	....	....	...	....	......	......
34.	F.	Mild tubercular leprosy..	+	+	+++++	+++++	......	......	......	......	....	....	...	....	......	......
35.	M.	Severe tubercular leprosy	+++	+++	++++	++++	......	......	......	......	....	....	...	....	......	......
36.	M.	Severe tubercular leprosy	++	++	++	++	......	......	......	......	....	....	...	....	......	......
37.	M.	Light tubercular leprosy	±	±	++	++	......	......	......	......	....	....	...	....	......	......
38.	M.	Severe tubercular leprosy	+++++	+++++	......	......	+++++	+++++	......	......	....	....	...	....	......	......
39.	F.	Light anesthesic leprosy	—	—	......	......	++++	++++	......	......	....	....	...	....	......	......
40.	F.	Light anesthesic leprosy	±	±	—	—	......	......	......	......	....	....	...	....	......	......
41.	M.	Severe anesthesic leprosy	—	—	......	......	......	......	......	......	—	—	...	....	......	......
42.	M.	Light tubercular leprosy	......	......	......	......	++++	++++	......	......	....	....	...	....	......	......
43.	M.	Light anesthesic leprosy	—	—	......	......	......	......	......	......	....	....	...	....	......	......
44.	M.	Light anesthesic leprosy	......	......	—	—	......	......	......	......	....	....	...	....	......	......
45.	M.	Light tubercular leprosy	......	......	++	++	......	......	......	......	....	....	...	....	......	......
46.	M.	Mild tubercular leprosy..	......	......	......	......	+++++	+++++	......	......	....	....	...	....	......	......
47.	M.	Syphilis (?)	—	—	+++	+++	—	—	—	—	....	....	—	—	—	—
48.	M.	Syphilis	—	—	++++	++++	—	—	—	—	....	....	—	—	......	......
49.	M.	Syphilis	—	—	++	+	—	—	—	—	....	....	—	—	......	......
50.	M.	Syphilis	—	—	++++	++++	++++	++++	—	—	....	....	—	—	......	......
51.	M.	Syphilis (?)	±	±	++++	++++	++++	++++	+	+	....	....	—	—	......	......
52.	M.	Gonorrheal epididymitis..	—	—	—	—	......	......	......	......	....	....	—	—	......	......
53.	M.	Tubercular arthritis (?)..	—	—	+++	+++	......	......	......	......	....	....	—	—	......	......
54.	F.	Syphilis	—	—	+++++	+++++	......	......	......	......	....	....	...	....	......	......

* The serum of this patient showed auto-inhibition.

By severe nodular leprosy is meant patients having numerous nodules in the face and other parts of the body; by mild, those who have lesser nodules in the body; by light, those who have only regional affection with but a few nodules or a number of the old nodules being absorbed, the illness being in a state of suspension for the present.

Under nervous leprosy, lepra maculosa is also included. The severity is determined either by the degree of deformity and emaciation in limbs, accompanied by difficulty of motion, or by cutaneous or muscular ulceration owing to the nutritive nervous difficulties, or general appearance of flecks.

+++++ = complete inhibition
++++ — nearly complete inhibition
+++ = strong inhibition
++ = mild inhibition
+ = weak inhibition
± = nearly complete lysis
— = complete lysis
M = masculine: F = feminine

The results of our experiments are given in Table 2. From Table 2 we shall summarize the effects produced by each antigen, separately, and make a critical study of the behavior of the serum.

Thirty-nine of the forty-two leprous sera examined gave unmistakable reactions (either positive or negative) with old tuberculin, 2 gave indistinct reactions, and 1 gave auto-inhibition. Positive and negative reactions are classified in Table 3 according to the type and the severity of the disease, in Table 4 according to severity only, and in Table 5 according to types.

TABLE 3

REACTIONS OF LEPROUS SERA WITH OLD TUBERCULIN

Cases	Number of Tests	Positive Results	Percentage of Positive Results
Severe tubercular leprosy	14	14	100.00
Mild tubercular leprosy	6	5	83.33
Light tubercular leprosy	4	2	50.00
Severe anesthesic leprosy	5	3	60.00
Mild anesthesic leprosy	3	0	0
Light anesthesic leprosy	7	3	42.85
Total	39	27	69.23

TABLE 4

REACTIONS OF LEPROUS SERA WITH OLD TUBERCULIN CLASSIFIED ACCORDING TO SEVERITY OF SYMPTOMS

Severity	Number of Cases	Positive Cases	Percentage of Positive Cases
Severe	19	17	89.48
Mild	9	5	55.56
Light	11	5	45.45

TABLE 5

REACTIONS OF LEPROUS SERA WITH OLD TUBERCULIN CLASSIFIED ACCORDING TO TYPES OF DISEASE

Type	Number of Cases	Positive Cases	Percentage of Positive Cases
Tubercular leprosy	24	21	87.50
Nervous leprosy	15	6	40.00

From Table 3 it is seen that a regular proportion exists between the reaction and the severity of the disease, when it is tubercular, while the same seems to apply in part to anesthesic leprosy. The reversed proportion that seems to exist in the light and mild forms must remain unexplained until more extensive tests can be made. It may possibly be due to the fact that latent non-anesthetic lesions existed in the internal organs of the body and that the patient showed only slight nervous disorder upon superficial examination. If the results of the test be classified according to the types of the disease, it is seen that the tubercular leprosy produced twice as many positive cases as the nervous leprosy, while the percentage increased regularly according as the severity of the disease was augmented.

Möller[1] recently reported results of complement fixation with leprous serum by the use as antigens of old tuberculin, new tuberculin, bovine tuberculin (prepared by Höchst), and the suspension of the pulverized human tubercle bacilli (laboratory preparation), according to the directions of Koch. An interesting comparison is made between his results and our own in Table 6.

TABLE 6

MÖLLER'S RESULTS IN COMPLEMENT-FIXATION WITH LEPROUS SERUM COMPARED WITH NAKAJO AND ASAKURA'S RESULTS

Facts	Möller's Results	Nakajo and Asakura's Results
Total number of tests	32	39
Positive reactions	25 (78.12%)	27 (69.23%)
Percentage positive		
(*a*) Tubercular leprosy	95-100	87.50
(*b*) Anesthesic leprosy	25	40.00
Relation	In direct proportion	In direct proportion

Our results seem to be inferior to Möller's in respect to the percentage positive of the test. However, this difference may be annihilated if only such of Möller's cases be given as produced a positive reaction with the culture fluid; that is, a total of 20 cases (60.25 percent). The singular reversal that occurred between the percentages of the tubercular and nervous cases may be explained by the difference of view regarding the diagnosis; for in the lighter forms these distinctions are often difficult to establish. The average percentage of both tubercular and nervous cases was 62.50 in Möller's case, while in ours it was slightly higher (63.75). The more the infection tends to be tubercular in nature, the more positive will be the results obtained.

Möller classified as positive such cases as brought about even a slight complement-fixation reaction, and we also classed the cases that showed a slight hemolytic inhibition among the positive cases.

In short, Möller's results and our own appear on the whole to agree with one another. The reactions go parallel with the severity of the symptoms and the quantity of micro-organisms in the patient's body;

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[1] Deutsch. med. Wchnschr., 1913, 39, p. 595.

for in tubercular leprosy a high ratio of positive reactions is obtained, while in nervous cases, in which only a few bacilli are found, which are regarded by various authors as degenerated forms of leprosy bacilli, the reaction test in question may be regarded as having a close relation to the very nature of the disease, altho the reaction is by no means specific with tuberculin. Our present test, however, may be compared in its clinical importance to Wassermann's test for syphilis made with the heart extract of a normal guinea-pig as antigen. With regard to the question suggested by Meyer, that is, whether tuberculin might not produce positive reactions not only in syphilis, but in other diseases, our test, altho made in but few cases, gave the results shown in Table 7.

TABLE 7
COMPLEMENT-FIXATION WITH OLD TUBERCULIN IN DISEASES OTHER THAN LEPROSY

Kinds of Disease	Positive Reaction	Negative Reaction	Para-reaction
Syphilis	—	4 cases	1 case
Tubercular arthritis	—	1 case	—
Gonorrheal epididymitis	—	1 case	—

Let us review the results obtained with Wassermann's syphilis test and the old tuberculin test of twenty-one leprous and eight non-leprous sera, respectively. (One leprous serum that gave auto-inhibition was excluded.)

From Table 8 we see that fourteen of twenty-one cases (two-thirds) gave positive results in both tests, while five of the remaining seven gave positive reactions with old tuberculin alone, and one of the remaining two gave a para-reaction with the former and a slight positive reaction with the latter. In short, our experiment shows that leprous sera give a much greater number of positive results in complement-fixation tests with old tuberculin than with Wassermann's syphilitic antigens; while with syphilitic sera just the reverse obtains. Thus, we may infer from the results of our experiments that the leprous sera contain properties that would give positive reactions to both antigens, and the syphilitic sera those that would give positive reactions to Wassermann's antigen only. The sera of the patient suffering from gonorrheal epididymitis did not react to either antigen.

We shall now give the results of our complement-fixation tests with leprous and non-leprous sera and the leprous nodule antigen and old tuberculin.

From Table 9 we may see that nine of seventeen cases produced positive results in both tests. Thirteen of seventeen cases gave homo-

TABLE 8

RESULTS OBTAINED WITH WASSERMANN'S SYPHILIS TEST AND THE OLD TUBERCULIN TEST

Kinds of Disease	Total Tests	Wassermann's Reaction			Complement-Fixation vs. Old Tuberculin			Number of Reactions Agreeing in Either Test
		Positive	Negative	Para-reaction	Positive	Negative	Para-reaction	
Severe tubercular leprosy..........	11	10	1	..	11	..	..	10
Mild tubercular leprosy...........	4	1	3	..	4	..	..	1
Light tubercular leprosy...........	2	1	1	..	1	..	1	..
Severe anesthesic leprosy..........	2	2	..	..	2	..	..	2
Mild anesthesic leprosy............	..	..	..	..	..	..	..	..
Light anesthesic leprosy...........	2	1	1	..	1	..	1	1
	21	15	6	..	19	..	2	14
Syphilis	4	4	..	..	..	4	..	..
Syphilis(?)	2	2	..	..	..	1	..	..
Tubercular arthritis(?)	1	1	..	..	..	1	..	..
Gonorrheal epididymitis	1	..	1	..	..	1	..	..
	8	7	1	..	..	7	..	..

The two cases of suspected syphilis and the one case of suspected tuberculosis (arthritis) were reported to be syphilis, while the gonorrheal epididymitis referred to us by Dr. Sugi, Chief of the Aomori Hospital, gave a negative Wassermann. These results agree with those obtained by us.

The two cases of light tubercular leprosy each alternately produced one instance of positive reaction; no coincident cases therefore occurred.

TABLE 9

COMPLEMENT-FIXATION TEST WITH LEPROUS AND NON-LEPROUS SERA AND THE LEPROUS NODULE ANTIGEN AND OLD TUBERCULIN

Kinds of Disease	Total Number of Tests	Complement-Fixation vs. Old Tuberculin			Complement-Fixation vs. Leprous Nodule Antigen			Number of Cases Agreeing	Number of Cases Not Agreeing
		Positive	Negative	Para-reaction	Positive	Negative	Para-reaction		
Severe tubercular leprosy	5	5	..	..	5	..	..	5	..
Mild tubercular leprosy	1	..	1	..	1	..	..	..	1
Light tubercular leprosy	2	1	1	..	1	1	..	2	..
Severe anesthesic leprosy	2	1	1	..	..	2	..	1	1
Mild anesthesic leprosy	2	..	2	..	..	2	..	2	..
Light anesthesic leprosy	5	2	3	..	2	3	..	3	2
	17	9	8	..	9	8	..	13	4
Syphilis	5	..	4	..	2	3	..	3	2

geneous results in both tests, seven of which were positive. Two of the remaining four cases that disagreed in both tests gave positive results with old tuberculin only, while the other two were positive with the leprous nodule antigen only. These positive reactions thus may be considered as being leprous in origin, for, as we saw in the second table, the leprous nodule antigen gave a strong positive reaction with syphilitic sera in two cases of five. Here, we wish to emphasize the importance of guarding against the production of a non-syphilitic positive reaction by testing the antigen with a number of non-syphilitic sera, for various acquired properties may be present in the organs that are to be used as antigens.

Let us now consider what is the relation existing between the results of the complement-fixation test of the leprous and of the non-leprous sera and the leprous positive clot antigen and old tuberculin.

TABLE 10

COMPLEMENT-FIXATION TEST WITH LEPROUS AND NON-LEPROUS SERA AND THE LEPROUS POSITIVE CLOT ANTIGEN AND OLD TUBERCULIN

Cases	Old Tuberculin		Positive Leprous Clot Antigen	
	Test-Tube 1	Test-Tube 2	Test-Tube 1	Test-Tube 2
1. M. Severe tubercular leprosy	+++	++	++++	++++
2. F. Severe tubercular leprosy	++	++	+++++	+++++
3. M. Severe anesthesic leprosy	++++	++++	+++++	+++++
4. F. Severe anesthesic leprosy	++	++	+	+
5. M. Light anesthesic leprosy	—	—	—	—
6. M. Light anesthesic tubercular leprosy	—	—	+	+
7. M. Mild anesthesic leprosy	—	—	—	—
8. M. Mild tubercular leprosy	++	+	+++++	+++++
9. F. Light anesthesic leprosy	—	—	—	—
10. Severe anesthesic leprosy	—	—	—	—
11. Mild anesthesic leprosy	—	—	—	—
12. M. Severe tubercular leprosy	+++++	+++++	+++++	+++++
13. M. Light nervous leprosy	+	+	+	+
14. M. Syphilis	—	—	—	—
15. M. Syphilis	—	—	—	—
16. M. Syphilis	—	—	—	—
17. M. Syphilis	—	—	—	—
18. M. Syphilis	±	±	+	+

From Table 10, it is seen that of thirteen cases, twelve gave homogeneous results in both tests, seven being positive and five negative. Of five cases of non-leprous patients (i. e., syphilitic), four agreed in both tests in being negative. There occurred no case showing non-conformity in the results. One instance of leprous serum that gave negative results with old tuberculin and positive with leprous positive clot antigen, and one of non-leprous serum that gave a para-reaction with old tuberculin and a slightly positive one with the leprous clot, constitute unimportant variations. These two materials, therefore, may be considered to have produced identical results. Moreover, strictly considered, the leprous positive clot showed some tendency to produce a non-leprous reaction because of the coinciding foreign properties that were found in the leprous nodule antigens.

Let us now consider the relation between the results obtained in the complement-fixation test with the leprous and non-leprous sera and the leprous negative clot antigen.

TABLE 11

COMPLEMENT-FIXATION WITH LEPROUS AND NON-LEPROUS SERA AND LEPROUS NEGATIVE CLOT ANTIGEN

Cases of Leprosy			Old Tuberculin		Leprous Negative Clot Antigen	
			Test-Tube 1	Test-Tube 2	Test-Tube 1	Test-Tube 2
1.	F.	Severe tubercular	++	++	—	—
2.	M.	Severe anesthesic	++++	++++	+	+
3.	F.	Severe anesthesic	++	++	—	—
4.	M.	Light anesthesic	—	—	—	—
5.	M.	Light anesthesic	+	+	—	—
6.	M.	Mild tubercular	—	—	—	—
7.	M.	Light tubercular	+++	+++	—	—
8.	M.	Mild anesthesic	—	—	—	—
9.	M.	Mild tubercular	—	—	—	—
10.	M.	Severe anesthesic	—	—	—	—

From Table 11, we see that only one case of the ten gave a slightly positive reaction with the leprous negative clot, all the other nine being negative, while five cases gave positive results with old tuberculin and no contradictory case occurred. Therefore, we may infer that the leprous negative clot contains little or no property that produces any reaction in the test in question; and that the leprous negative clot produces no conforming results with old tuberculin in the leprous complement-fixation test different from those produced by the leprous positive clot.

We shall now give the results of the tests made with syphilitic blood clot and old tuberculin and leprous and non-leprous sera.

TABLE 12
COMPLEMENT-FIXATION TEST WITH LEPROUS AND NON-LEPROUS SERA, THE SYPHILITIC BLOOD CLOT AND OLD TUBERCULIN BEING USED AS ANTIGENS

Cases			Old Tuberculin		Non-Leprous Clot Antigen	
			Test-Tube 1	Test-Tube 2	Test-Tube 1	Test-Tube 2
1.	M.	Severe tubercular leprosy	+++	++	—	—
2.	F.	Severe tubercular leprosy	++	++	—	—
3.	M.	Severe anesthesic leprosy	++++	++++	—	—
4.	F.	Severe anesthesic leprosy	++	++	—	—
5.	M.	Light anesthesic leprosy	—	—	—	—
6.	M.	Light tubercular leprosy	—	—	—	—
7.	M.	Severe tubercular leprosy	+++++	+++++	—	—
8.	M.	Light tubercular leprosy	+++	+++	—	—
9.	M.	Mild anesthesic leprosy	—	—	—	—
10.	M.	Light tubercular leprosy	—	—	—	—
1.	M.	Syphilis	—	—	—	—
2.	M.	Syphilis	—	—	—	—
3.	M.	Syphilis	—	—	—	—
4.	M.	Syphilis	—	—	—	—
5.	M.	Syphilis	±	±	—	—
6.	M.	Gonorrheal epididymitis	—	—	—	—
7.	M.	Syphilis	—	—	—	—

From Table 12, we may conclude that non-leprous clot posesses no property like that of leprous positive clot, for the non-leprous clot antigen produced negative results with all the sera of ten lepers, as well as with that of seven non-leprous patients, while tuberculin reacted positively in six of the ten cases.

Finally, we shall compare the results obtained in the tests made with guinea-pig blood clot and old tuberculin on leprous and non-leprous sera.

TABLE 13

COMPLEMENT-FIXATION TEST WITH LEPROUS AND NON-LEPROUS SERA, GUINEA-PIG BLOOD CLOT AND OLD TUBERCULIN BEING USED AS ANTIGENS

Cases			Old Tuberculin		Blood Clot of Guinea-Pig	
			Test-Tube 1	Test-Tube 2	Test-Tube 1	Test-Tube 2
1.	M.	Severe tubercular leprosy	+++	++	++++	++++
2.	M.	Mild anesthesic leprosy	—	—	—	—
3.	M.	Mild tubercular leprosy	++	+	+++++	+++++
4.	F.	Light anesthesic leprosy	—	—	—	—
5.	M.	Severe anesthesic leprosy	—	—	—	—
6.	M.	Syphilis	—	—	—	—

Tho our test was made on a small number of cases, the results in both instances agreed perfectly.

This report concerns the complement-fixation tests made upon the sera of forty-six lepers and eight non-leprous patients, the total number of tests being 155.

SUMMARY

Our results in complement-fixation tests with leprous sera and old tuberculin, considered from the standpoint of the type of the disease, show that a higher percentage positive, that is, 87.5 percent, was obtained in tubercular leprosy, than was obtained in nervous leprosy—40 percent. Classified according to the severity of the symptoms, these results are: 89.48 percent positive cases, severe; 55.56 percent, mild; and 45.45 percent, slight. From these facts, we may conclude that the reaction produced by old tuberculin bears a direct relation to the nature of leprosy, tho, as Babes[4] and Möller[1] affirm, it is never specific. Moreover, the heart extract of a guinea-pig may be substituted for it in the same way as in the Wassermann[2] test.

As Meier[3] has pointed out, if there is any doubt as to the disease (whether syphilis or leprosy) from which a patient is suffering, it will probably prove to be leprosy if the complement-fixation tests with both old tuberculin and Wassermann's[2] antigen give positive results. If the test reacts positively only with old tuberculin, the disease is unmistakably leprosy; but negative results are not sufficient to exclude the case as leprosy.

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2. Hemolysine, Zytotoxine und Praezipitine, 1910, p. 79.
3. Verhandl. d. 2. Internat. Leprakonferenz in Bergen, 1909, 3, p. 334.
4. Ztschr. f. Immunitätsf., 1910, 7, p. 578.

Altho no difference occurred between the percentages of the positive cases in complement-fixation tests with leprous sera and either old tuberculin or leprous nodule, cases nevertheless occurred in which, not only were the results obtained in both tests reversed, but in certain syphilitic sera the leprous nodule antigen brought about a considerably large number of positive results. This fact appears to us to be explained on the ground that these results are non-specific in their nature, if the term "specific" be applied in its wider sense, inasmuch as the heart extract of a guinea-pig can be said to produce a "specific" reaction to syphilis. These facts led us to conjecture that this positive result may have been a reaction, not against the disease primarily present in the sera, but against certain other co-existing properties, a case in which the ratio of the genuinely positive results would have been less than that appearing in our table (see Table 9).

The clot of leprous blood, the sera of which reacted positively with leprous nodule antigen (leprous positive clot antigen), and the blood clot from a guinea-pig brought about identical results, as did the old tuberculin in complement-fixation tests with leprous sera; but we regret that they were tested in too few cases to enable us to draw any conclusions therefrom. The leprous negative clot antigen, which was obtained from the blood giving no complement-fixation with leprous nodule antigen, and the clot from a syphilitic patient, which gave negative results with the leprous nodule antigen, gave almost negative results, that is, identical with those produced in non-leprous (viz., syphilitic) sera. It seems, therefore, that these antigens contain properties differing from those of the other two antigens. Closer observation, moreover, reveals the fact that there were some cases that gave a very slight positive reaction with leprous positive clot antigen, as well as with the negative clot antigen; for this reason we deem it necessary to continue the study of these two kinds of antigens.